Conclusions: Increased levels of VEGF165 protein synthesis are detected in the cells stimulated with Il-1β. Both GS and CS inhibited the increase in this protein induced by the stimulation, while NSAIDs were unable to modify VEGF165 presence. Our data suggests that NSAIDs and SYSADOAs could have a different profile in controlling the presence of angiogenic mediators in the OA cartilage.

179 ANTIAPOPTOTIC EFFECT FOR CHONDROCYTE BY N-ACETYLCYSTEINE

S. Nakagawa, Y. Arai, O. Mazda, S. Fujita, K. Honjo, N. Hiraoka, K.A. Takahashi, J. Imanishi, T. Kubo. *Kyoto Prefectural University of Medicine, Kyoto, JAPAN*

Purpose: Articular cartilage in human osteoarthritis (OA) has more apoptotic chondrocytes than that of normal cartilage, and apoptosis has been considered to play an important role in the development of OA. It is reported that increased oxidative stress reduces chondrocyte survival. N-acetylcysteine (NAC) is a thiol, a mucolytic agent, a precursor of L-cysteine and reduced gluthathione, and scavenger of ROS. Chondrocytes are very sensitive to ROS, but can survive in the presence of L-cysteine. The objective of this study is to examine the effect of anti-apoptotic effect for chondrocyte by NAC.
Methods: The isolated chondrocytes of male japanease white rabbit were cultured and the culture medium was replaced with presence or absence of NAC. Sodium nitroprusside dehydrate (SNP) which is generates nitric oxide (NO) was added to the culture medium for induction of apoptosis, and the cells were cultured for 12hours. For DNA visualization, chondrocytes were stained with 2% Hoechst 33342. The cells were observed under a fluorescence microscope. Chondrocyte apoptosis was detected by staining with TdT-mediated dUTP nick end labeling (TUNEL). Amount of intercellular ROS was also evaluated. To ascertain wether glutathione is involved, inhibition assay was performed by using glutathione synthetase inhibitor, buthionine sulfoximine (BSO).
Results: In the NAC-untreated group with SNP, a generous amount of chodrocytes exhibited typical characteristics of apoptosis with highly fragmented, condensed or divided nuclei. In contrast, fewer apoptotic cells observed in the NAC-treated culture. The positive rate of apoptotic cell with SNP addition reduced significantly by NAC treatment. Amount of intercellular ROS was elevated transiently by SNP treatment, but decreased significantly by NAC. By the treatment of BSO the effect of NAC was disappeared in all assays.
Conclusions: Those studies showed that ROS is involved in NO toxity. NAC appeared to have remarkable protecting effect in chondrocytes subjected to NO via glutathione synthesis. NAC has been used for treatment of acetaminophen toxicity in clinical practice, and its safeness has already confirmed. In this study, These finding supports the possibility that NAC could prevent the progression of OA through the suppression of apoptosis.

180 CD90 AND CD166 REPRESENT PREDICTIVE MARKERS OF EARLY CHONDROCYTES DEDIFFERENTIATION

S. Giovannini, J. Diaz-Romero, P. Mainil-Varlet, D. Nesic. *Institute of Pathology, Bern, SWITZERLAND*

Purpose: Treatments of cartilage defect with tissue-engineering techniques rely on the chondrogenic capacities of isolated and culture-expanded cells. During expansion in monolayer, human articular chondrocytes (HAC) lose their chondrogenic potential and become dependent on differentiation factors for neochodrogenesis. In this study, the interconnection between dedifferentiation process, cell proliferation and intrinsic/chondrogenic factors-dependent redifferentiation capacities was established. Moreover, changes in the surface marker expression during the early expansion phase was correlated with the intrinsic chondrogenic capacites.
Methods: Hyaline cartilage samples were collected post-mortem from human knee joints. After isolation, HAC were expanded in DMEM-F12 medium containing 10% FBS. Redifferentiation potential of HAC was assessed in micromass pellets culture incubated for 3 weeks in high glucose DMEM serum free media without (−T−D) or with (+T+D) TGF-β1 (10 ng/ml) and dexamethasone (10^{-7} M). Proteoglycan synthesis was evaluated via Safranin-O staining and collagen type II production via immunohistochemistry. Cellularity and the glycosaminoglycans content of the pellets were quantified using CyQuant kit and dimethylmethylene blue dye binding assay respectively. Surface marker expression was monitored during early expansion phase in monolayer using flow cytometry.
Results: The requirement for chondrogenic factors was analyzed in pellets produced by HAC expanded for 10 days (primary) and for 5 weeks (passaged) HAC. Pellets from primary HAC cultured without the addition of chondrogenic factors produced neocartilage tissue with extracellular matrix rich in proteoglycans and collagen type II. In contrast, in pellets from passaged HAC, the presence of both factors proved necessary for neochondrogenesis. Thus, −T−D condition was identified as optimal to assess HAC intrinsic chondrogenic potential. To identify when during expansion HAC lose their intrinsic chondrogenic potential, cells were expanded from 1 to 6 weeks in monolayer, and pellets were prepared. Cell expansion corresponding to less than 3.2±0.4 cumulative population doublings (PD) resulted in the formation of neocartilage tissue in −T−D condition. In contrast, cells that underwent more than 3.2±0.4 cumulative PD produced only fibrous-like tissue indicating complete loss of intrinsic chondrogenic potential. Chondrogenesis could be restored by the addition of chondrogenic factors (+T+D). The GAG/DNA analysis indicated that HAC expanded for 1 week produced higher amounts of GAGs in −T−D compared to +T+D condition, suggesting inhibitory effect of chondrogenic stimuli in the early expansion phase. However, already after 2 weeks of expansion, the addition of chondrogenic stimuli had beneficial effect. To investigate whether changes in surface marker expression could be related to the early dedifferentation process, several surface markers were analyzed. A marked upregulation of CD90 and CD166 was observed after 10 days expansion in monolayer culture.
Conclusions: Loss of HAC intrinsic chondrogenic capacities in vitro appears to be a continuous process which correlates with the proliferation rate of HAC, identifying 3.2±0.4 PD as a critical number of cell divisions. The progressive loss of intrinsic redifferentiation potential can be partly reversed by the presence of chondrogenic factors TGF-β1 and dexamethasone as early as 14 days expansion. Upregulation of CD90 and CD166 after 10 days of expansion represents predictive marker of early HAC dedifferentiation before complete loss of intrinsic chondrogenic potential occurs.

181 SALMON CALCITONIN INCREASE PROTEOGLYCAN FORMATION FORMATION IN HUMAN OA ARTICULAR CARTILAGE

B-C. Sondergaard, S.H. Madsen, C. Christiansen, M.A. Karsdal. *Nordic Bioscience, Herlev, DENMARK*

Purpose: Calcitonin has previously been demonstrated to have chondroprotective effects on articular cartilage under *in vivo* experimental conditions. It is debated whether this effect is exclusively due to effect on subchondral bone remodelling or in part due to direct effects on the articular cartilage. We investigated possible direct anabolic effects of calcitonin in human OA articular cartilage with focus on cartilage formation of proteoglycans and collagen type II.
Methods: Human OA cartilage was obtained from knee arthroplasty operations, which was dissected into 12−14 mg explants and cultured in six replicates with refreshment of medium every second or third day in the presence or absence of salmon calcitonin [0.01pM-100 nM] in DMEM:F12 supplemented with 2% Ultrose G. As positive control other cartilage explants were stimulated with 100 ng/mL IGF and as negative control for cell-mediated effects metabolic inactivated cartilage explants were used. Direct effects of calcitonin on articular cartilage was evaluated using (1) following proteoglycan synthesis by radioactive labeled $^{35}SO_4$ [5 μCi] after 18 days of culture and extracting the proteoglycans by 4 M GuHCl treatment, (2) investigations of metabolic activity using the cell viability assay AlamarBlue, (3) collagen type II formation measured as neoepitopes of propeptides of collagen type II was quantified by the PIINP ELISA.
Results: Calcitonin significantly ($P < 0.01$) and concentration-dependently [0.01 pM-100 nM]. Induced proteoglycan synthesis measured by radioactive sulphate incorporation with a 40% maximal induction at 10 nM, corresponding to the levels of the positive control 100 ng/ml IGF. In alignment, calcitonin treatment concentration-dependently [0.01 pM-100 nM] resulted in significant ($P < 0.01$) 35% increased levels of collagen type II synthesis.
Conclusions: Calcitonin treatment increased proteoglycan and collagen synthesis of human OA cartilage. Calcitonin may provide benefit to the management of joint diseases via the direct effects on chondrocytes in addition to the well-established osteoclast mediated effects on subchondral bone.